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^{1}H, ^{13}C and ^{15}N NMR backbone assignments of 25.5 kDa metallo-β-lactamase from *Bacteroides fragilis*

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Biological context

Antibiotic resistance in the significant human pathogen *Bacteroides fragilis* is manifest through the production of metallo-β-lactamase (CcrA). Mature CcrA (residues 18–249) hydrolyzes the lactam ring of traditional β-lactam-based antibiotics such as the penicillins, and possesses a binuclear zinc site (Concha et al., 1996) with a molecular weight of 25.5 kDa. Currently, there are no known inhibitors for CcrA in clinical use. We have undertaken an investigation of the solution structure and dynamic properties of CcrA using NMR spectroscopy. This information will be used to guide the development of potent metallo-β-lactamase inhibitors.

Methods and results

The gene encoding for residues 18–249 of CcrA was expressed in *Escherichia coli* BL21(DE3) cells (Yang et al., 1992) in a modified minimal media preparation. Metallo-β-lactamase preparations were assayed using nitrocefin (Oxoid), and yielded kinetic values consistent with previously published results (Yang et al., 1992).

NMR spectra were acquired at 295 K on Bruker AMX-500, -600 and DRX-600 spectrometers equipped with triple resonance probes. All triple resonance experiments were performed on either ^{13}C,^{15}N- or ^{2}H,^{13}C,^{15}N- uniformly labeled CcrA. ^{15}N-HSQC, ^{15}N-edited TOCSY-HSQC and NOESY-HSQC experiments were performed on ^{15}N-uniformly labeled CcrA. All samples were prepared in 10 mM HEPES buffer, pH 7.0, with 0.01% NaN_3 in 95% H_2O/5% D_2O and 10 μM excess $ZnCl_2$. The crystal structure of CcrA shows that both zinc sites are occupied when an identical excess of $ZnCl_2$ was used (Concha et al., 1996).

Triple resonance experiments which were found to be useful for backbone assignments included the HNCO, HNCA, HN(CO)CA (Grzesiek and Bax, 1992b), HN(CA)CB, HN(COCA)CB (Yamazaki et al., 1994), CBCA(CO)NH (Grzesiek and Bax, 1992a), HCACO (Zhang and Gmeiner, 1996), HN(CA)H (Seip et al., 1992) and C(CO)NH (Grzesiek et al., 1993) experiments. A detailed account of experimental conditions will be described elsewhere.

Experiments with uniformly ^{13}C,^{15}N-labeled CcrA were performed to establish sequential assignments. These were commenced with the HNCO experiment which allowed all observable spin systems to be identified. Both the CBCA(CO)NH and C(CO)NH experiments were used to group interresidue ($i - 1$) spin systems into specific amino acid types. The combined use of HNCA and HCACO experiments allowed H^{α} and C^{α} nuclei to be correlated to intraresidue carbonyl and backbone amide groups. The HCACO experiment was also used to confirm and extend H^{α} (i) chemical shift assignments made through the combined use of HN(CA)H and ^{15}N TOCSY-HSQC experiments. This approach allowed only 40% of sequential backbone assignments to be completed.

Since the normally useful HNCACB experiment (Wittekind and Mueller, 1993) exhibited poor signal-to-noise, a triple-labelled sample of CcrA was used to acquire HN(CA)CB and HN(COCA)CB spectra, allowing over 84% of $C^{\beta}(i-1)/C^{\beta}(i)$ pairs to be identified. However, extensive sample degradation during

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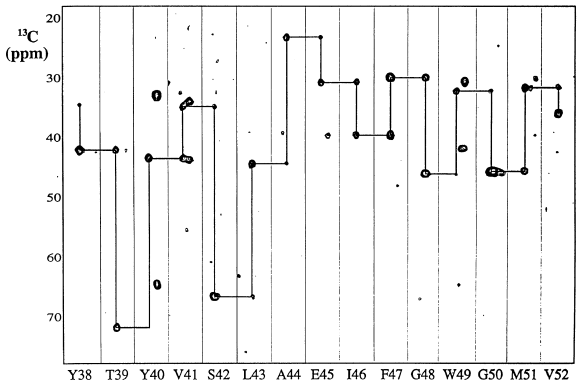

Figure 1. Strip plot of the HN(CA)CB spectrum of ^{2}H,^{13}C,^{15}N-uniformly labeled CcrA displaying $^{13}C^{\beta}$ $(i-1)$/$^{13}C^{\beta}$ (i) correlations to intraresidue ^{15}N and HN chemical shifts for residues 38–52. Negative cross peaks are observed for correlations involving the $^{13}C^{\alpha}$ nucleus of glycine residues.

acquisition precluded the correlation of the remaining pairs.

Initial attempts to assign histidine and cysteine residues associated with the active site were largely unsuccessful. Of the eight histidine residues in CcrA, histidines 99, 101, 162 and 223 are involved in coordinating the two zinc atoms (Concha et al., 1996). According to the X-ray structure (Concha et al., 1996), CcrA possesses three cysteine residues, one of which binds a zinc ion (C181) and a second is located in close proximity to the active site (C104). ^{15}N-HSQC spectra, acquired using separate ^{15}N-His and -Cys labeled CcrA samples, were instrumental in identifying all corresponding resonances. The addition of a fourfold excess of EDTA resulted in the immediate loss of signal for the resonances of residues associated with the active site, whereas only slight shifts were observed for the remaining signals. These observations will be discussed in detail elsewhere. Since the random coil chemical shift for the reduced cysteine C^{β} nucleus (28 ppm) moves approximately 13 ppm downfield upon oxidation, it can be used as a very sensitive probe to the oxidation state of the sulfide (Wishart et al., 1995). The C^{β} chemical shift for each cysteine in CcrA is indicative of the reduced form.

A database containing all available inter- and intraresidue $^{1}H^{\alpha}$, $^{13}C^{\alpha}$, $^{13}C^{\beta}$ and ^{13}CO chemical shift data for each spin system was created. Since it was not possible to obtain complete chemical shift information for several spin systems, searches were made by correlating intraresidue chemical shifts of at least two nuclei with the corresponding interresidue nuclei of other spin systems. In cases where several candidates were possible, the ^{15}N-edited NOESY-HSQC experiment was used to identify sequential or long-range HN-HN or H^{α}-HN connectivities.

Extent of assignments and data deposition

The assignment of ^{15}N and HN resonances for 214 of 220 (97.3%) possible backbone amides was determined. Resonances for the remaining residues were either not detected or there were no unambiguous connectivities in triple resonance experiments. For the ^{13}C nuclei, 97.8% of C^{α}, 98.1% of C^{β} and 97.4% of CO resonances were assigned. A total of 91.4% of H^{α} resonances were assigned specifically. Chemical shift assignments have been deposited in the BioMagResBank database (accession no. 4102).

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